



Aposematic coloration and mimicry in opisthobranch mollusks: new phylogenetic and experimental data

Terrence M. Gosliner

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ABSTRACT Expression of aposematic color patterns in opisthobranchs is more prevalent and more overtly manifested in more derived rather than more basal taxa. This is the case in clades such as *Thuridilla*, *Siphopteron*, *Flabellina* and *Halgerda*. In other instances, one taxon is cryptic and its sister taxon exhibits aposematic coloration. Generally, closely related species have similar color patterns, but in one clade of *Flabellina*, four closely related species have strongly divergent color patterns. Sympatric species with similar color patterns result from both descent from a common ancestor as well as from convergent evolution. Contemporary phylogenetic analytical methods provide a basis for distinguishing evolution of similar color patterns between common descent and homoplasy. Species-specific differences in palatability of similarly colored prey for different predators blur traditional distinctions between Batesian and Müllerian mimicry. Additional studies on other mimicry complexes to determine whether these patterns are more generalized need to be undertaken.

RIASSUNTO L'espressione di pattern cromatici aposematici negli opistobranchi sembra essersi maggiormente affermata in taxa "derivati" piuttosto che "basali". Questo è evidente in cladi quali *Thuridilla*, *Siphopteron*, *Flabellina* and *Halgerda*. D'altra parte, un taxon può presentare una colorazione criptica e la sua "specie sorella" avere una colorazione aposematica. Di norma, specie filogeneticamente affini hanno pattern cromatici simili, ma in un clade di *Flabellina*, quattro specie strettamente imparentate mostrano una colorazione fortemente divergente. Specie simpatiche, pur presentando una colorazione simile, possono derivare sia da un antenato comune che essere la conseguenza di un'evoluzione convergente. Gli attuali metodi d'analisi filogenetica permettono di distinguere se un pattern cromatico derivi da un processo evolutivo diretto o sia dovuto ad un fenomeno di convergenza. Differenze specie-specifiche tra specie a pattern cromatici simili nell'appetibilità da parte di predatori diversi rendono meno chiara la tradizionale distinzione tra mimetismo batesiano e mulleriano. Nuovi studi su altri complessi mimetici saranno comunque necessari per definire se tali processi siano più generali di quanto ritenuto fino ad oggi.

T. M. GOSLINER Department of Invertebrate Zoology and Geology, California Academy of Sciences, Golden Gate Park, San Francisco, California 94118 U.S.A e-mail: tgosline@calacademy.org

INTRODUCTION

Opisthobranch mollusks have been shown to be excellent study organisms for illustrating the phenomenon of aposematic coloration (ROS, 1977; EDMUNDS, 1987, 1991; GOSLINER & BEHRENS, 1990; RUDMAN, 1991). Although Edmunds questioned whether aposematic coloration as an evolutionary strategy has been adequately demonstrated in opisthobranchs, GOSLINER & BEHRENS (*op. cit.*) suggested that the criteria for demonstration had been met. Studies on the chemical nature of secondary metabolites (FAULKNER & GHISELIN, 1983; CIMINO & GHISELIN, 1998, 1999; AVILA, 1995) strongly support the idea that opisthobranchs have employed sequestered chemical compounds as a primary defensive strategy. Utilization of chemical defense mechanisms by opisthobranchs represents a major divergence from other heterobranchs and other gastropod taxa where the molluscan shell provides the primary defense against predation (VERMEIJ, 1978). Species-level phylogenetic hypotheses of various opisthobranch taxa recently have provided new data by which evolution of color patterns in various opisthobranch lineages may be examined (GOSLINER & KUZIRIAN, 1990; GOSLINER & WILLAN, 1991; GOSLINER & JOHNSON, 1994, 1999; GOSLINER, 1995; FAHEY & GOSLINER, 1999). Recent studies of opisthobranchs in tropical regions have demonstrated that opisthobranchs and other organisms, especially polyklad flatworms, have similar color patterns and potentially form mimicry complexes (GOSLINER & BEHRENS, 1990). Little evidence has

been presented to suggest whether these organisms with similar color patterns are palatable to predators and whether they are differentially palatable to various potential predators. These issues relate directly to the question of whether these apparent mimicry complexes are functional with regard to predator/prey interactions and whether they represent cases of Batesian or Müllerian mimicry. This paper presents evidence of the nature of color pattern differentiation in several distantly related clades of opisthobranchs to determine the array of evolutionary strategies found in opisthobranchs. Similarly, results from field and laboratory feeding experiments using similarly colored opisthobranchs and flatworms as experimental subjects are presented.

MATERIAL AND METHODS

Phylogenetic studies were undertaken employing the methodology described in individual papers that are cited. In general, morphological data sets were analyzed using versions of PAUP (Phylogenetic Analysis Using Parsimony) (SWOFFORD, 1993). In some instances (e.g., GOSLINER & JOHNSON, 1999), distinctive color data were included in the construction of the original phylogenetic hypotheses, while in other cases (GOSLINER & WILLAN, 1991) they were not. In each case discussed, the basic assumptions about inclusion or exclusion of characters related to color are explicitly stated. In all cases where color characters were included, tree topology was not markedly altered from the case where these char-



acters were excluded. Rather, greater resolution of polytomies within clades resulted. Field observations of attempted predation were undertaken by direct observation using mask and snorkel in shallow-water habitats and SCUBA for deeper-water situations. Laboratory experiments focused primarily on eliciting feeding responses on opisthobranchs using a variety of predators. Experimental subjects included the nudibranch species *Chromodoris annae* Bergh, 1877, *C. magnifica* (Quoy & Gaimard, 1832), *Phyllodesmium briareum* (Bergh, 1896) and *Flabellina riwo* Gosliner and Willan, 1991 as well as the sacoglossan *Plakobranchus* sp. All specimens of these species were collected in the vicinity of Madang Lagoon, Papua New Guinea during 4-11 August 1989. In other cases, predation experiments were conducted upon a nudibranch/flatworm pair where both individuals share a similar color pattern. These experiments focused primarily upon the nudibranch *Chromodoris preciosa* (Kelaart, 1858) and the polyclad flatworm *Pseudoceros* sp. (Fig. 1A). Specimens of both species were observed in their natural habitat in 1-2 meters of water in Kranket Lagoon, Madang, Papua New Guinea on 12 August 1989. Individuals of both species were abundant and were found within 5-10 cm of each other. Individuals of *Pseudoceros* sp. were approximately five times as abundant as specimens of *Chromodoris preciosa*. Specimens of both species were collected by hand and were transferred to the laboratory at the Christensen Research Institute where they were maintained in 90 litre aquaria. Experimental subjects for predation experiments were conducted by collecting single individuals of four species of common reef fishes from the Christensen Research Institute dock at Nagada Harbour, Madang. Fish were collected from Nagada Harbour rather than from Kranket Lagoon to diminish the likelihood that individuals had previously encountered specimens of the two potential prey. Neither *Pseudoceros* sp. nor *Chromodoris preciosa* had been observed from the area where the fish were caught, despite repeated observation of nudibranch and flatworm populations at that locality. The four species of predators were the pallid triggerfish, *Sufflamen bursa* (Bloch & Schneider, 1801), the silty wrasse, *Halichoeres purpurascens* (Bloch & Schneider, 1801), Jansen's wrasse, *Thalassoma janseni* (Bleeker, 1856) and the honeycomb cod, *Epinephelus merra* Bloch, 1793. All of these species are generalists that feed on a variety of small benthic prey. These fish were collected by hook and line and transferred to marine aquaria and were allowed to acclimate to laboratory conditions for 2-3 days. During the predation experiments, individual fish were isolated in the aquaria to which they had been acclimated. Polyclad or nudibranch prey were placed into the top of each aquarium by emptying a small volume of seawater containing the individual potential prey. Fish behavior was then recorded and nudibranchs and polyclads that were not consumed after five minutes were then removed to their storage aquaria. Fish were then fed small pieces of squid or bread and were not fed again until the following day's experiments had been completed. The same individual fish were then utilized for the subsequent days' experiments.

RESULTS

Evolution of aposematic coloration:

Various lineages of opisthobranchs appear to have evolved representatives that exhibit aposematic coloration. For example, the majority of sacoglossan opisthobranch clades contain animals with greenish coloration and are clearly associated with and cryptic upon specific algal prey (JENSEN, 1997). Most basal lineages contain taxa that are exclusively whitish or green in color. Some representatives of sacoglossan clades have bright, colorful pigment that is in striking contrast to that of their algal prey. For example, *Cyerce nigricans* (PEASE, 1866) is black with bright orange and blue iridescent markings that clearly make it stand out from its grassy green algal prey, *Chlorodesmis fastigiata*. A few sacoglossans within the Limapontiidae are brightly colored in contrast to their prey. Most Plakobranchiidae have a greenish body color and often have contrasting parapodial margins. Within *Thuridilla* Bergh, 1872, a few species have a greenish body color while most others have brightly colored bodies with bright iridescent pigment spots and parapodial marginal lines (GOSLINER, 1995). Behaviorally, individuals of different species of *Thuridilla* are often seen crawling out in the open on shallow patch reefs. Rather than feeding on relatively large fleshy algae as is most common for species of *Elysia* Risso, 1818 (JENSEN, *op. cit.*), species of *Thuridilla* appear to feed upon small clumps of finely filamentous algae (GAVAGNIN *et al.*, 1994). Phylogenetic studies of *Thuridilla* indicate that all basal species have a green body color, while the majority of derived taxa have a bright, iridescent body. The construction of the phylogenetic hypothesis for *Thuridilla* included color characters (GOSLINER, *op. cit.*). Removal of these characters yielded a similar tree topology, but with less resolution. In both instances the most basal taxon was *T. carlsoni* Gosliner, 1995. This species lacks contrasting pigment spots or brightly colored parapodial marginal lines. *Thuridilla carlsoni* has a white marginal band that is somewhat contrasting, but is similar to that found in species of *Elysia*. *Thuridilla kathae* Gosliner, 1995 and *T. flavomaculata* Gosliner, 1995, are also basal taxa with green body color and white or cream marginal bands that are not strikingly contrasting on normal rocky substrates. One basal taxon, *T. multimarginata* Gosliner, 1995, has a green body color but has contrasting parapodial bands of reflective orange, blue and black lines. The only other species with green body color (*T. picta* (Verrill, 1901), *T. decorata* Heller and Thompson, 1983 and *T. indopacifica* Gosliner, 1995) all have brightly colored marginal lines. The remaining species all have brightly colored body pigment and other bright, contrasting pigment on the body, such as marginal parapodial lines or spots.

Sister species of *Thuridilla* have color patterns that are similar in appearance. For example, *T. gracilis* (Risbec, 1928) (as *T. bayeri* (Marcus, 1965) and *T. splendens* (Baba, 1949) both have dark brown to black bodies with cream longitudinal lines (GOSLINER, 1995: figs. 1a,b, e). Both species often have reflective blue pigment, but that pigment may be absent in some specimens of *T. gracilis*. *Thuridilla undula* Gosliner, 1995 and *T. lineolata* (Bergh, 1905) are sister species with a light blue body with parapodial bands of bright orange and a submarginal line of



black (GOSLINER, *op. cit.*: figs. 1d, 29a). In the former, the bands undulate while in the latter they are straight. In the species pair of *T. livida* (Baba, 1955) and *T. boffae* Gosliner, 1995 both have a black body with an orange marginal line. In *T. livida* there is a reflective blue submarginal line while in *T. boffae* there are blue patches (GOSLINER, *op. cit.*: figs. 1c, 16f).

The only species-level phylogeny for cephalaspidean opisthobranchs is that of the Gastropteridae (GOSLINER, 1989). Within the genus *Sagminopteron* Tokioka & Baba, 1964, the four species are subdivided into two clades, one of which contains two species with coloration that is cryptic on their prey sponges and the other two that are brightly colored on their prey. The two species with aposematic coloration, *S. psychedelicum* Carlson & Hoff, 1974 and *S. ornatum* Tokioka & Baba, 1964, have bright color patterns that are strikingly dissimilar. Species of the sister taxon, *Siphopteron* Gosliner, 1989 are all brightly colored and assumed to exhibit aposematic coloration, with the exception of the most basal Indo-Pacific taxon, *S. pohupei* Hoff and Carlson, 1983, which blends in with a generalized background. Species of *Enotopteron* Minichev, 1967 are generally cryptic while species of *Gastropteron* Meckel in Kosse, 1813 may be either drably or brightly colored. The only species-level phylogenies of aeolidoidean nudibranchs are those for the Flabellinidae (GOSLINER & KUZIRIAN, 1990; GOSLINER & WILLAN, 1991). Construction of these phylogenies did not incorporate any data related to coloration or pigment patterns. Within the aeolidoidean taxon, Flabellinidae, basal species have a translucent white body color with a brown or red digestive gland that is visible through the transparent epithelium of the cerata. Often contrasting opaque white rings are present at or immediately proximal to the ceratal apices. Some more derived taxa, including *F. lineata* (Lovén, 1846), *F. capensis* (Thiele, 1925), *F. trilineata* (O'Donoghue, 1921) and *F. cooperi* (Cockerell, 1901) have additional opaque white lines on the dorsal and lateral sides of the body. Many more highly derived taxa have an orange or purple rather than white body color, including members of five distinct clades. The first clade contains two described species, *F. cynara* (Marcus & Marcus, 1967) and *F. iodinea* (Cooper, 1863). The second clade contains several taxa with papillate rhinophores: *F. delicata* Gosliner & Willan, 1991, *F. exoptata* Gosliner & Willan, 1991, *F. marcusorum* Gosliner & Kuzirian, 1990, *F. rubrolineata* (O'Donoghue, 1929), *F. poenicia* (Burn, 1957), *F. arveloi* Ortea & Espinosa, 1998 and *F. hammami* Gosliner, 1994. Many members of this clade also have subapical purple markings on the cerata. The third clade contains species with annulate rhinophores: *F. affinis* (Gmelin, 1791), *F. funeka* Gosliner & Griffiths, 1981 and *F. ischitana* Hirano & Thompson, 1990. In the cases of *F. pedata* (Montagu, 1815) and *F. rubropurpurata* Gosliner & Willan, 1991, they are the only members of their respective clades with a purple body color. Members of one subclade of *Flabellina* Voigt, 1834 containing four species all have opaque white markings on the translucent white body and cerata. In these species there is a subapical band of brightly colored pigment. However, the color patterns of these species, all of which occur sympatrically in the Philippines, are markedly different. *Flabellina macassarana* Bergh, 1905 and *F. bicolor*

(Kelaart, 1858) have diffuse white pigment scattered uniformly over the surface of the body. The cerata have an orange subapical band that is darker and more diffuse in *F. macassarana*. In contrast, *F. riwo* Gosliner & Willan, 1991, has an intricate network of opaque white on the body and a purple subapical band. *Flabellina bilas* Gosliner & Willan, 1991 has alternating opaque white mid-dorsal diamonds and red and opaque white subapical ceratal bands. The clade of nudibranchs that contains the greatest number of species is the Doridoidea. Within this large taxon are two major lineages, the Phanerobranchia and the Cryptobranchia. Cryptobranchs are the most diverse in terms of numbers of species. Many of the cryptobranchs are cryptic in their coloration while other subclades predominantly contain species that exhibit aposematic color patterns. GOSLINER & JOHNSON (1994) demonstrated that the Chromodorididae and Actinocyclusidae are sister taxa. The two clades exhibit dramatically different evolutionary patterns. Members of the Actinocyclusidae lack defensive glands and are exclusively cryptically colored. All species thus far studied have color patterns that closely resemble the sponges on which they feed. The entire clade contains 14 species of *Hallaxa* Eliot, 1909 and two species of *Actinocyclus* Ehrenberg, 1831. There appears to be little correlation between the color pattern and phylogeny within *Hallaxa*. It is evident that species with white body color and opaque white markings evolved on at least three separate occasions in *H. translucens* Gosliner & Johnson, 1994, *H. paulinae* Gosliner & Johnson, 1994 and *H. cryptica* Gosliner & Johnson, 1994.

In contrast to the Actinocyclusidae, the chromodorids exhibit very different patterns of evolution. Virtually all species of chromodorids have defensive mantle glands located around the periphery of the mantle. The species where glands are absent appear to have secondarily lost glands rather than primitively lacked them (GOSLINER & JOHNSON, 1999). While the Actinocyclusidae contains only 16 described species, their sister taxon, Chromodorididae, contains more than 600 described species. In this instance, evolution of defensive mantle glands can be considered a key innovation that has contributed to the extensive radiation and speciation that has occurred in this lineage. Almost all species within this clade exhibit aposematic color patterns. Chromodorids are among the most brightly colored of shallow-water marine organisms. Species of the chromodorid clade, *Hypselodoris* Stimpson, 1855, exhibit distinct patterns of pigmentation within different subclades (GOSLINER & JOHNSON, *op. cit.*). Construction of *Hypselodoris* phylogenetic hypotheses included color pattern data. To test the impact of these data on overall phylogenetic pattern, color data were then removed from a second series of analyses. As in the case of *Thuridilla*, tree topology was conserved, but resolution was lost. Members of the clade that is found in the Atlantic and eastern Pacific Oceans have a bluish body color while members of the their sister Indo-Pacific clade generally have a translucent white body, but exhibit far more variability. The four members of the clade restricted to the eastern Pacific all have dark blue pigment with a pattern of bright yellow spots. Other aspects of their color pattern, such as marginal bands and the presence of blue spots are more divergent between species. In some Atlantic

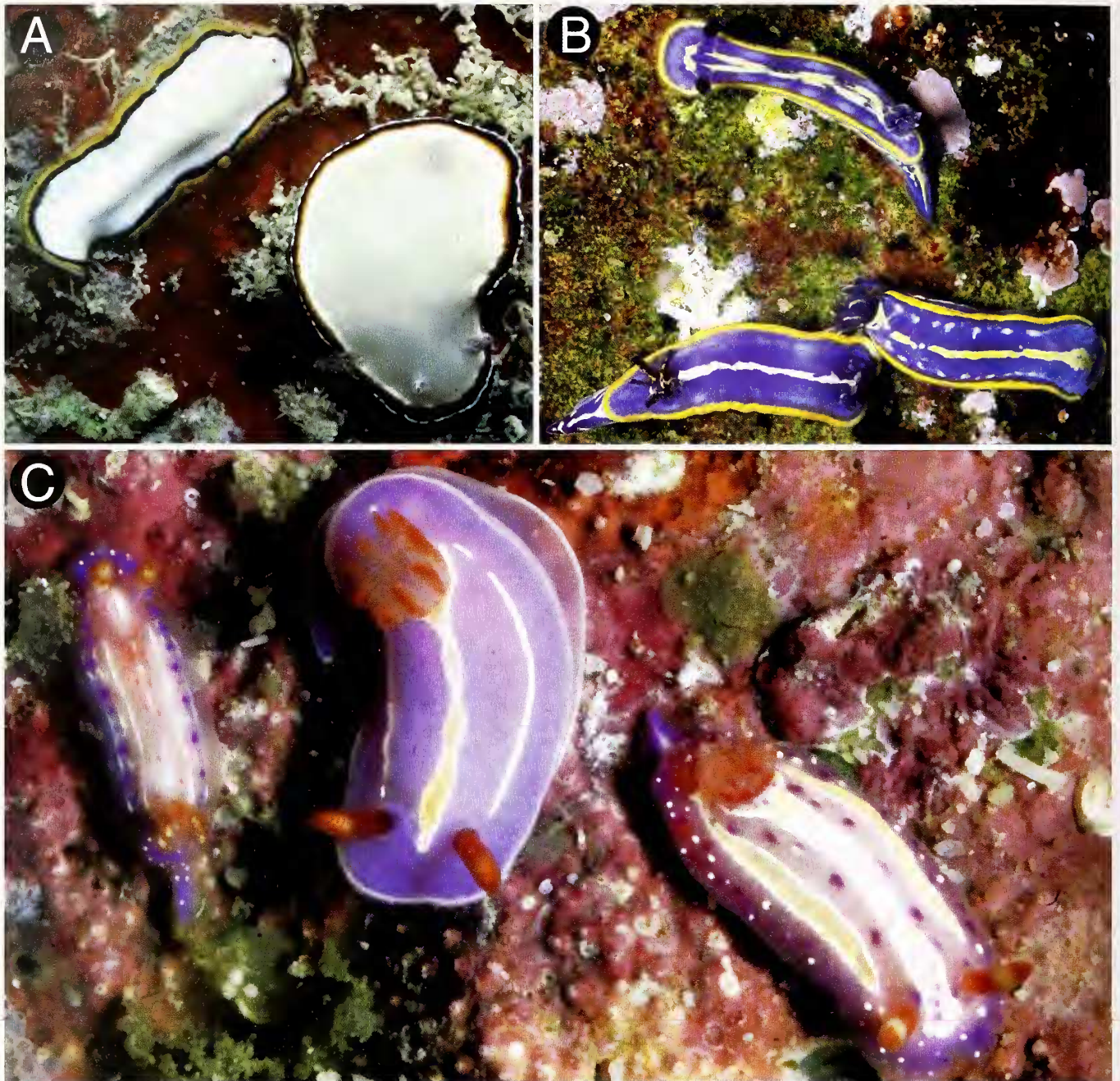


Figure 1. A. Polyclad and nudibranch mimics, left, *Pseudoceros* sp., right, *Chromodoris preciosa* (Kelaart, 1858). B. Similarly colored species of *Hypselodoris* found together at Isla Tarifa, Straight of Gibraltar, Asturias, Spain, upper, *H. bilineata* (Pruvot-Fol, 1953), lower left, *H. fontandraui* (Pruvot-Fol, 1951), lower right, *H. midatlantica* Gosliner, 1990. C. Similarly colored opisthobranchs collected within one meter of each other, southern Luzon, Philippines, left, *Hypselodoris maculosa* (Pease, 1871), middle, *Pectenodoris trilineata* (Adams & Reeve, 1850), right, *P. aurora* Johnson & Gosliner, 1998.



regions, such as the Strait of Gibraltar, as many as five or six species may be sympatric. All sympatric species share a bright blue body color with yellow lines and/or spots (Figure 1B). Within one clade of Indo-Pacific taxa, all members have a series of longitudinal lines of pigment. In the more basal members these lines are purple, while in more highly derived species the lines are opaque white. Most of the species in this clade are endemic to the Hawaiian Islands. One of these taxa, *H. alboterminata* Gosliner & Johnson, 1999, has violet lines. The remaining Hawaiian taxa that are members of this clade, *H. peasei* (Bergh, 1880) (as *H. andersoni*), *H. violabranhia* Gosliner & Johnson, 1999 and *H. insulana* Gosliner & Johnson, 1999, all have opaque white longitudinal lines. Another Hawaiian endemic, *H. bertschi* Gosliner & Johnson, 1999 is a member of another subclade of Indo-Pacific taxa. This species also has opaque white longitudinal lines despite the fact that its closest relatives lack them. The remaining two taxa found in the Hawaiian Islands, *H. infucata* (Rüppell & Leuckart, 1828) and *H. paulinae* Gosliner & Johnson, 1999 lack any hint of opaque white lines. Two white-lined sister species are also found in the western Indian Ocean. These two taxa, *H. carnea* (Bergh, 1889) and *H. capensis* (Barnard, 1927) are geographically separated. The former species is found from the Red Sea to tropical southern Africa while the latter is restricted to temperate waters of southern Africa. Sympatric with *H. carnea* in tropical southern Africa is another similarly colored species, *H. fucata* Gosliner & Johnson, 1999, which is a member of another clade. *Hypselodoris fucata* is the only member of its clade that possesses opaque white lines. The chromodorid genus *Pectenodoris* Rudman, 1984 is known from two species, *P. trilineata* (Adams & Reeve, 1850) and *P. aurora* Johnson & Gosliner, 1998. Both species are sympatric in the Philippines. *Pectenodoris trilineata* has a purple body with three longitudinal yellow or white lines. The rhinophores are uniformly red with a purple base. In contrast, *P. aurora* has a pale pink body color with purple pigment near the margins. Mid-dorsally it has three cream colored longitudinal bands surrounded by an opaque white line. There are scattered opaque white and black spots over the surface of the notum and the rhinophores are white with alternating red and white bands. The pigment pattern is very similar to that of *Hypselodoris maculosa* (Pease, 1871), which is also sympatric with the two species of *Pectenodoris*. The photo shown in Figure 1C depicts specimens of these three species all collected within a meter of each other in southern Luzon, Philippines. Members of the genus *Halgerda* Bergh, 1880 are generally brightly colored doridoideans found only in the Indo-Pacific tropics and adjacent warm temperate regions. A preliminary phylogeny of these taxa was constructed (FAHEY & GOSLINER, 1999) using color data in addition to anatomical characters. Subsequent analyses, removing color data, produced the same tree topology but yielded poorer resolution within some clades, as has been documented for *Tburidilla* and *Hypselodoris*. Basal taxa, such as *Halgerda dalangbita* Fahey & Gosliner, 1999 and *H. paliensis* (Bertsch & Johnson, 1982), have a more uniform and drab body color. More derived taxa have dorsal ridges that are punctuated with bright pigment that is highly contrasting with the general body

color (FAHEY & GOSLINER, *op. cit.*). One clade, which contains species that possess distinctive reproductive anatomy, includes six species, *H. aurantiomaculata* (Allan, 1932), *H. batangas* Carlson & Hoff, 2000, *H. carlsoni* Rudman, 1978, *H. diaphana* Fahey & Gosliner, 1999, *H. malesso* Carlson & Hoff, 1993 and *H. terrantuentis* Bertsch & Johnson, 1982. All of these species have bright orange pigment on the notal ridges or tubercles and small black spots on the surface of the gills and rhinophores. Some of these may have additional secondary orange spots or lines over the surface of the dorsum.

Predator/prey feeding experiments

Feeding experiments were initially conducted with individual nudibranchs with different species of fishes. These nudibranchs were chosen because they represented different color patterns and some appeared to be cryptic in appearance while others are brightly colored. Specimens of two brightly colored dorid nudibranchs, *Chromodoris magnifica* and *C. annae*, were offered to individuals of four different species of fishes, Jansen's wrasse, a silty wrasse, a pallid triggerfish and a honeycomb cod. Neither of these nudibranchs was attacked by any of the fishes. After a period of five minutes, a specimen of the aeolid nudibranch, *Phyllodesmium briareum*, which is normally cryptic on its soft coral prey, was offered to the same predator individuals. The silty wrasse and the honeycomb cod avoided the nudibranch. The Jansen's wrasse mouthed and rejected the nudibranch. The nudibranch crawled away unharmed. The pallid triggerfish ate one specimen of *P. briareum* and regurgitated several cerata. Immediately following ingestion, the triggerfish flared its gills and opened and closed its mouth rapidly for 30–60 seconds. After approximately five minutes, it was offered a second individual of *P. briareum*. It ingested it and regurgitated several cerata. The gills and mouth exhibited the same reaction as with the first nudibranch. After another five minutes elapsed, the triggerfish was offered a third specimen of *P. briareum*. The nudibranch was closely scrutinized by the triggerfish but there was no attempt to ingest this individual. The following day, all four species were offered specimens of *Phyllodesmium briareum*. All species avoided any feeding attempts of the nudibranchs and specimens of the cryptically colored sacoglossan, *Plakobrancheus* sp., but readily consumed pieces of squid and small chunks of bread. A specimen of the brightly colored aeolid, *Flabellina riwo*, was offered to all four fishes. It was avoided by all potential predators, with the exception of the pallid triggerfish. The triggerfish ingested one specimen and regurgitated it in a damaged condition. The triggerfish was offered a second specimen of *F. riwo*. It ingested the nudibranch, but immediately regurgitated it unharmed. The triggerfish avoided a third specimen of *F. riwo*. On day three, all four species of fish avoided specimens of *Phyllodesmium briareum*, but readily consumed squid and bread. No further experiments were conducted with specimens of *Plakobrancheus* sp. or *Flabellina riwo*. On day 4, specimens of the polyclad flatworm, *Pseudoceros* sp., were offered to the Jansen's wrasse, the pallid triggerfish and the honeycomb cod. The wrasse and triggerfish each ingested the polyclad and immediately regurgitated it unharmed. They avoided additional flatworm speci-



mens offered to them. The honeycomb cod readily ingested the specimen of *Pseudoceros* sp. It was immediately offered three more individuals of the same species of flatworm, each of which was readily ingested. The same cod was then offered a specimen of the nudibranch, *Chromodoris preciosa*, which has a color pattern that is very similar to the polyclad. The cod readily ingested the nudibranch and rapidly regurgitated it unharmed. Its gills flared and its mouth was held open and quivering for approximately five minutes. After it appeared to recover, the cod was offered an additional specimen of the nudibranch. It avoided the nudibranch. It was subsequently offered additional specimens of the similarly colored polyclad, which it also avoided. The same cod specimen was offered the same species of polyclad and nudibranch for each of the next seven days. It avoided both potential prey on all subsequent occasions, but continued to consume squid and bread.

DISCUSSION

Color patterns in opisthobranch gastropods are varied in their evolutionary history. In many instances, the more basal members of clades are more drably colored and cryptic than the more derived taxa. Within the sacoglossan genus *Tburidilla*, more basal species have a green body color without markedly contrasting parapodial margins. More derived members of this clade have brightly colored, reflective pigment with contrasting parapodial margins. Within the cephalaspidean taxon, Gastropteridae, the subclade of *Siphopteron* found in the Indo-Pacific region, the most basal taxon is cryptically colored while the remaining, more derived species are all aposematically colored. Flabellinid aeolid nudibranchs also exhibit a pattern where more basal taxa are less brightly colored than more derived ones. More derived species have additional opaque white lines on the dorsal surface and often have brightly colored rather than translucent white bodies. More basal members of the dorid nudibranch genus *Halgerda* are cryptically colored while all derived taxa are aposematic in their color patterns. In the majority of clades studied thus far, the more derived taxa can be considered to be more brightly colored than their less derived ancestors. Unfortunately, corresponding data about the relative toxicity of these taxa are not currently available. It would not be unexpected however to find a positive correlation between increased distastefulness and greater use of aposematic coloration in more derived members of clades.

In a few cases, some clades contain only cryptically colored species while their sister taxon contains only aposematically colored representatives. One subclade of the cephalaspidean taxon *Sagaminopteron* contains two species that are aposematically colored while its sister subclade includes two species that are cryptic. Two major sister clades of cryptobranch dorids are the Actinocyliidae and Chromodorididae. The former contains all cryptic taxa while the latter includes some of the most vividly colored of all nudibranchs and virtually all members are brightly colored. These sister clades also exhibit very different patterns of adaptive radiation. The Actinocyliidae contains only 16 species worldwide, while its sister taxon has more than 600 described species. It has been hypothesized (GOSLINER &

JOHNSON, 1994) that these differences in adaptive radiation and color patterns relate directly to the presence of a key innovation in the Chromodorididae, the presence of defensive mantle glands. Another general aspect of the evolution of color patterns that exhibits variation, is that closely related or sister species tend to have similar color patterns. The exception is found in closely related species of the species rich taxon, *Flabellina*, which tend to have divergent color patterns in members of the same clade. Here the pattern of opaque white pigment and color of ceratal bands varies considerably between species. Most other opisthobranch taxa that have been studied appear to have inherited elements of their color pattern from their common ancestor. In *Tburidilla*, sister species tend to have similar color patterns. This pattern is also evident for species of the aposematic dorid taxa *Hypselodoris* and *Halgerda*. The evolution of color patterns is especially well documented in *Hypselodoris* (GOSLINER & JOHNSON, 1999). In this clade there is a strong positive correlation between biogeography, phylogeny and similarity of color pattern. The two major sister groups are geographically vicariant with one occurring in the Atlantic and eastern Pacific and the other occurring only in the Indo-Pacific. Atlantic and eastern Pacific taxa all have a blue body color. Even within this clade, the eastern Pacific taxa, which represent a monophyletic subclade, all have very similar color patterns that are distinct from the Atlantic taxa.

In the Indo-Pacific subclade, there are superb examples of the relationship between evolution of a common pattern within a clade and phylogeny. Members of one clade all have opaque white longitudinal lines on the dorsal surface of the body. The most parsimonious explanation of this color pattern is that it was inherited from a single common ancestor. Sympatric with these white-lined species are additional species that have white lines. However, phylogenetic analysis indicates that these taxa are not as closely related to the white-lined species as they are to other species that lack white lines. Thus, these color patterns appear to have evolved convergently. At a single locality there may be sympatric congeneric species that have evolved similar color patterns from a common ancestor as well as others that represent cases of convergence. We also see evidence that other closely related taxa at a lower degree of relationship have examples of convergence when they are found sympatrically. Sister species of *Pectenodoris* have some elements of the same color pattern that is likely inherited from their common ancestor, but the convergence between *P. aurora* and the more distantly related *Hypselodoris maculosa* is striking. Convergent evolution is also found frequently in taxa that are far more distantly related, such as nudibranchs, flatworms and holothurians (GOSLINER & BEHRENS, 1990).

One would expect that sister species that were vicariantly separated would have similar color patterns at the time of divergence of their populations. However one would not expect these patterns to persist for long periods of time, if allopatry continues and these taxa are living outside of the context of individual predators that can learn to recognize the similarity of their color pattern. It is surprising that some of the deeply-based phylogenetic color patterns have persisted for significant



periods of time. In the absence of a fossil record, it is virtually impossible to know how old some of these clades of soft-bodied animals might be. However, some circumstantial evidence is illuminating. For example, we know that the eastern Pacific clade of *Hypselodoris* has all of its closest relatives in the Atlantic, not in other parts of the Pacific. We also know that these taxa have been isolated from their Atlantic ancestors since the closure of the Isthmus of Panama some 3.5 million years ago. Yet, the basic blue body with yellow pigment has persisted in the eastern Pacific members of the clade. There have been some modifications in the pattern in that the blue is darker and the yellow is in the form of spots rather than lines in all Pacific members of this clade.

Feeding experiments on various nudibranchs and polyclads by different predators demonstrate several consistent behavioral patterns. Most "naïve" fish that are generalists on varied small benthic prey attempted to feed on nudibranchs when they were offered. Most fishes regurgitated the nudibranchs or portions of the body in the case of some cerata-bearing nudibranchs. Generally, these encounters left the nudibranch unharmed. This fact suggests that individual selection is strongly favored as an explanation for the evolution of aposematic coloration rather than having to invoke group selection models, which have been strongly criticized (ROSEMBERG, 1989; TULLROT & SUNDBERG, 1991). WIKLUND & JARVI (1982) have suggested individual selection as the primary method of adaptation for aposematically colored insects that survive attacks by birds. Another conclusion from the feeding experiments is that predators learn rapidly to associate unpleasant encounters of attempting to feed on nudibranchs with the specific color patterns exhibited by the particular slug species. The learning experience and subsequent avoidance of nudibranch prey persisted for at least the entire time that the experiments were conducted, which in one instance was up to a week in duration.

Other conclusions about the evolution of mimicry can be drawn from the experiments involving similarly colored specimens of the polyclad flatworm, *Pseudoceros* sp., and *Chromodoris preciosa*. In two cases the predators regurgitated specimens of the polyclad and avoided the nudibranchs. The third species of fish, the honeycomb cod, readily ate the polyclads but rejected the nudibranch. Subsequently, the fish avoided both the nudibranchs and the polyclads. This suggests a classic Batesian mimicry scenario where the model (*C. preciosa*) is unpalatable and the mimic (*Pseudoceros* sp.) is palatable. A complicating factor to this hypothesis is that the mimic is found in greater densities in the natural habitat than the model. One possible explanation of how this mimicry can be stable stems from the fact that the polyclad is also unpalatable to two of the three remaining fish tested. This suggests that in the case of multiple predators, these two potential prey species actually represent cases of Müllerian mimicry, since both are likely to be unpalatable. The situation occurring with *Pseudoceros* sp. and *Chromodoris* sp. blurs the distinction between the classification of similarly colored taxa as strictly Batesian or Müllerian mimics. If the distinction is species-specific for different predators found sympatrically, it is suggestive that differences in relative palata-

bility vary on a continuous gradient rather than as absolutes.

Evolution of aposematic color patterns in opisthobranchs exhibits generalized patterns of becoming more prevalent and more overtly manifested in more derived rather than more basal taxa. Sympatric species with similar color patterns exist as products of both inheritance from a common ancestor as well as from convergent evolution. Utilization of contemporary phylogenetic analytical methods permits the distinction of color pattern evolution between common descent and homoplasy. Species-specific differences in palatability for different predators of similarly colored species complexes blur traditional distinctions between Batesian and Müllerian mimicry. Future studies on evolution of color patterns and mimicry in opisthobranchs should correlate these findings with chemical data and other tests for toxicity and relative palatability. This exercise will provide valuable data for determining whether more brightly colored species are in fact more distasteful to predators.

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LITERATURE CITED

- AVILA, C. 1995. Natural products of opisthobranch molluscs: a biological review. *Oceanography and Marine Biology: an Annual Review*, Aberdeen, 33:487-559.
- CIMINO, G. & GHISELIN, M., 1998. Chemical defense and evolution in the Sacoglossa (Mollusca: Gastropoda: Opisthobranchia). *Chemoecology*, Stuttgart, 8:51-60.
- CIMINO, G. & GHISELIN, M., 1999. Chemical defense and evolutionary trends in biosynthetic capacity among dorid nudibranchs (Mollusca: Gastropoda: Opisthobranchia). *Chemoecology*, Stuttgart, 9:187-207.
- EDMUNDS, M. 1987. Color in opisthobranchs. *American Malacological Bulletin*, Austin, 5(2):185-196.
- EDMUNDS, M. 1991. Does warning coloration occur in nudibranchs? *Malacologia*, Philadelphia, 32(2):241-255.
- FAHEY, S. & GOSLINER, T.M., 1999. Preliminary phylogeny of *Halgerda* (Nudibranchia: Halgerdidae) from the tropical Indo-Pacific, with descriptions of three new species. *Proceedings of the California Academy of Sciences*, San Francisco, 51 (11): 425-448.
- FAULKNER, D. J. & GHISELIN, M.T., 1983. Chemical defense and evolutionary ecology of dorid nudibranchs and some other opisthobranch gastropods. *Marine Ecology. Progress Series*, Amelinghausen, 13 (2-3):295-301.



- GAVAGNIN, M., MARIN, A., MOLLO, E., CRISPINO, A., VILLANI, G. & CIMINO, G. 1994. Secondary metabolites from Mediterranean Elysioidae: origin and biological role. *Comparative Biochemistry and Physiology*, New York, 108B: 107-115.
- GOSLINER, T. 1989. Revision of the Gastropteridae (Opisthobranchia: Cephalaspidea) with descriptions of a new genus and six new species. *The Veliger*, Berkeley, 32(4): 333-381.
- GOSLINER, T. 1995. The genus *Thuridilla* (Opisthobranchia: Elysiidae) from the tropical Indo-Pacific, with a revision of the phylogeny and systematics of the Elysiidae. *Proceedings of the California Academy of Sciences*, San Francisco, 49(1): 1-54.
- GOSLINER, T. & BEHRENS, D.W. 1990. Special resemblance, aposematic coloration and mimicry in opisthobranch gastropods. In: M. Wicksten (Ed). *Adaptive Significance of Color in Invertebrates*. Texas A. & M. Univ. Press: College Station. pp. 127-138
- GOSLINER, T. M. & JOHNSON, R.F. 1999. Phylogeny of *Hypselodoris* (Nudibranchia: Chromodorididae) with a review of the monophyletic clade of Indo-Pacific species, including descriptions of twelve new species. *Zoological Journal of the Linnean Society*, London, 125:1-113.
- GOSLINER, T. M. & KUZIRIAN, A.M. 1990. Two new species of Flabellinidae (Opisthobranchia: Aeolidacea) from Baja California. *Proceedings of the California Academy of Sciences*, San Francisco, 47(1):1-15.
- GOSLINER, T. M. & JOHNSON, S. 1994. Review of the genus *Hallaxa* (Nudibranchia: Actinocyclusidae), with descriptions of nine new species. *The Veliger*, Berkeley, 37(2): 155-191.
- GOSLINER, T. M., & WILLAN, R.C. 1991. Review of the Flabellinidae (Nudibranchia: Aeolidacea) from the tropical Indo-Pacific, with the descriptions of five new species. *The Veliger*, Berkeley, 34(2):97-133.
- JENSEN, K. 1997. Evolution of the Sacoglossa (Mollusca, Opisthobranchia) and the ecological associations with their food plants. *Evolutionary Ecology*, London, 11:301-335.
- JOHNSON, R. F. & GOSLINER, T.M. 1998. The genus *Pectenodoris* (Nudibranchia: Chromodorididae) from the Indo-Pacific, with the description of a new species. *Proceedings of the California Academy of Sciences*, San Francisco, 50 (12):295-306.
- ROS, J. 1977. La defensa en los opisthobranchios. *Investigación y Ciencia*, Barcelona, 12:48-60.
- ROSEMBERG, G. 1989. Aposematism evolves by individual selection: evidence from marine gastropods with pelagic larvae. *Evolution*, Boulder, 43: 1811-1813.
- RUDMAN, W. B. 1991. Purpose in pattern: the evolution of colour in chromodorid nudibranchs. *Journal of Molluscan Studies*, Oxford, 57 (supplement):5-21.
- SWOFFORD, D. 1993. *Phylogenetic Analysis Using Parsimony. Version 3.1*. Washington: Laboratory of Molecular Systematics. Smithsonian Institution.
- TULLROT, A. & SUNDBERG, P. 1991. The conspicuous nudibranch (*Polycera quadrilineata*): aposematic coloration and individual selection. *Animal Behavior*, London, 41: 175-176.
- VERMEIJ, G. 1978. *Biogeography and Adaptation: Patterns of Marine Life*. Harvard University Press.
- WIKLUND, C. & JARVI, T. 1982. Survival of distasteful insects after being attacked by naive birds: a reappraisal of the theory of aposematic coloration evolving through individual selection. *Evolution*, Boulder, 36: 988-1002.